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Persistence of erythromycin resistance gene *erm*(B) in cattle feedlot pens over time‡

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ABSTRACT

Antibiotic resistance in food animals has become an important issue for public health safety. The genes that code antibiotic resistance often enter the feedlot environment via feces and have the potential to be transferred through agroecosystems and into the food chain, either directly in their original bacterial host or via horizontal gene transfer. The objective of this study was to determine the distribution of erythromycin resistance genes associated with beef cattle excretions and ascertain whether these genes are enriched in areas of feedlot pens with high deposition of fecal material over time. The spatial distribution of manure accumulation was determined using georeferenced electromagnetic induction (EMI) readings at two times and EMI directed soil sampling. Feedlot surface samples from high- and low-manure accumulation zones were compared. The data indicated that 14 months of manure accumulation did not result in an increase in *erm*(B) positive feedlot soils, and the distribution of *erm*(B) genes was not correlated with areas of high manure deposition within the pens.

Keywords: Antibiotic resistance, resistance, antibiotic resistance gene, manure, erythromycin, *erm*B, feedlot pen, cattle, PCR, food animals

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INTRODUCTION

Erythromycin, a macrolide antibiotic commonly used to treat infections in humans, is on the World Health Organization's list of antimicrobial agents that are critical to human health (World Health Organization, 2007). Related macrolides (Tulathromycin (Draxxin), Tilmicosin (Micotil), and Tylosin (Tylan)) have been used in cattle to treat respiratory disease, pneumonia, metritis, mastitis, and foot rot (Smith Thomas, 2009). Tylosin is also used as a feed additive for cattle to prevent liver abscesses, and as part of a mineral supplement to help control pinkeye (Smith Thomas, 2009). Bacteria can develop resistance to macrolide antibiotics by encoding a suite of more

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than 30 erythromycin ribosomal methylase (*erm*) genes (Roberts *et al.*, 1999). The *erm* genes can be found in both commensal and pathogenic bacteria, including Gram-positive and -negative species (De Leener *et al.*, 2004; Roberts, 2004; Bae *et al.*, 2005; Chen *et al.*, 2007; Dogan *et al.*, 2005).

In bacteria from cattle, resistance to tylosin is encoded by *erm* genes and *erm*(B) is the most common (Jost *et al.*, 2004). For example, in a survey of U.S. livestock systems, the *erm*(B) gene was found to represent 56% of the total *erm* genes in bovine manure samples (Chen *et al.*, 2007). The *erm*(B) gene has been reported in *Campylobacter* from cattle feedlots (Bae *et al.*, 2005), Enteroccocci from pastured cattle (Anderson *et al.*, 2008), in livestock manure and pre-harvest production systems (Chen *et al.*, 2007), and pen floor fecal samples from feedlot heifers (Jacob *et al.*, 2008). Many studies screen for the *erm*(B) gene from pathogenic and commensal bacterial isolates, but this strategy does not allow for the assessment of non-target, unculturable bacteria. Since one of the primary concerns associated with antibiotic resistance in agricultural settings is the horizontal gene transfer from animals to humans, whole community DNA needs to be screened in order to assess the entire reservoir of antibiotic resistance genes present in a sample (Isaacson and Torrence, 2002). One element that contributes to human health risk associated with antibiotic resistance genes from agricultural settings is the persistence of the genes over time (Unc and Goss, 2004). A longitudinal study demonstrated that the *erm*(B) gene could persist in fecal samples from cattle in field conditions for over 150 days (Alexander *et al.*, 2011).

In commercial cattle feedlot operations, feces are continually deposited onto the pen surface and accumulate until they are removed by scraping, typically once a year. Identification of zones within the feedlot that are enriched for antibiotic resistance genes would allow for targeted sampling and remediation efforts. The large size and spatial heterogeneity of the feedlot pen presents challenges for sample collection. Typically, cattle in pens tend to congregate in certain areas, resulting in zones of high manure accumulation in the pen. Previous studies identified correlations between electromagnetic induction (EMI) readings and areas of high manure deposition (Woodbury *et al.*, 2009; Eigenberg *et al.*, 2010).

We hypothesized that the incidence of *erm*(B) genes in the feedlot were a consequence of excretion from the animal and would be concentrated in areas with high manure enrichment. To test this hypothesis we examined cattle feedlot pens that were allowed to accumulate manure for 14 months. Feedlot pen surface samples were collected based on differences in manure accumulation, delineated using EMI sampling methods and were evaluated using a conventional PCR-based *erm*(B) assay of total community DNA samples.

MATERIALS AND METHODS

Sampling

In order to ensure that samples were collected from areas representing the continuum of manure deposition, pens were mapped for EMI and sample sites were co-located with selected EMI values using the spatial response surface sampling design (RSSD) program contained in the USDA-ARS ESAP (EC_e Sampling Assessment and Prediction) software package (Lesch *et al.*, 2000).

Feedlot surface material samples were taken from ten feedlot pens (each 30 m by 60 m) at the U.S. Meat Animal Research Center, in conjunction with a previously described study (Spiehs *et al.*, 2012). Half of the pens contained animals receiving a normal, controlled diet of dry-rolled corn and half of the pens contained animals receiving a diet containing 14 – 35% wet distillers grains plus solubles (WDGS) (levels changed based upon the age of the cattle in the pens). All pens have a concrete apron adjacent to the feeding area and water areas along the lateral sides of the pens with a mound in the center. Following EMI mapping, twelve sample sites were identified in each pen, as described above, and GPS coordinates were recorded. In general, the feedlot pens have a gradient slope at 2% declination from the feeding area down to the bottom of the pen

where aged manure and liquids accumulate (Woodbury *et al.*, 2009). During the 13-month study, the pens were not cleaned and two separate groups of cattle were fed in the pens, each containing 32 mixed-breed finishing steers per pen. The first set was exchanged for the second between September 18th and 22nd, 2009.

A total of 240 feedlot surface material samples were collected (120 from June 2009, 120 from August 2010). Grab samples of the feedlot surface material were collected from the surface (0 to 10 cm depth), placed in 3.8-L plastic bags, and held on ice during transport to the laboratory. Aliquots of the samples were stored at -20°C until DNA extractions could be performed. The remaining feedlot surface material was immediately dried in a forced-air oven at 100°C for 24 hours, ground, and analyzed for moisture content, volatile solids content, nutrients, and soil pH as previously described by Spiehs *et al.* (2012).

DNA Extraction and Quantification

Feedlot surface soil samples were extracted as previously described by Miller *et al.* (1999), and purified using a Wizard® DNA Purification System (Promega, Madison, WI). DNA concentrations were determined using fluorometry. Calibration standards were created using diluted λ DNA (Quant-iT™ PicoGreen® dsDNA Assay Kit) at concentrations of 1 μg mL^{-1}, 10 μg mL^{-1}, 100 μg mL^{-1}, and 1000 μg mL^{-1}, and PicoGreen® was diluted to 1:200 with 1xTE. The standards, mixed with the diluted PicoGreen®, were used to make a linear standard curve for calibration. Samples were prepared by mixing 5 μL of sample, 45 μL of 1xTE, and 50 μL of diluted PicoGreen®. Samples were allowed to rest under aluminum foil for 5 minutes and then the fluorescence was measured. To verify fluorometric results, a subset of samples (three samples from each set of 30) was also screened on 1.5% agarose gels using established mass standards. Gels were stained for 10 minutes in an ethidium bromide solution, destained for 25 minutes in distilled water, and visualized on a UV transilluminator (Ultraviolet Productions, Upland, CA).

Polymerase Chain Reaction

A polymerase chain reaction (PCR) assay was performed for the detection of the *erm*(B) gene using primers developed by Böckelmann *et al.* (2009). The forward and reverse primer sequences were 5′-GGATTCTACAAGCGTACCTTGGA-3′ and 5′-GCTGGCAGCTTAAGCAATTGCT-3′, respectively. The amplification reactions were made with 0.25 μL each of forward and reverse primers (1:100 concentration), 11 μL PCR grade water, 12.5 μL Jumpstart Red TAQ ReadyMix (Sigma-Aldrich, St. Louis, MO), and 1 μL diluted sample (1:100 concentration). Positive and negative controls were run for every assay. Thermocycling was performed using a PTC-100 Peltier thermocycler (Bio-Rad, Hercules, CA). The cycles were set at 95°C for 2 minutes, then 35 cycles repeating through 95°C for 30 seconds, 60°C for 45 seconds and 72°C for 1 minute, then finally 72°C for 7 minutes. Samples were run on an agarose gel for 45 minutes on 145 V, stained with ethidium bromide for 10 minutes, and then destained with distilled water for 20 minutes. Gels were photographed using a Kodak Gel Logic 100 Imaging System (Carestream Health, Inc., Rochester, NY).

Statistical Analysis

The ANOVA and Logistic procedures available in SAS Analysis program version 9.2 (SAS Inst., Cary, NC) were used to determine the effect of diet treatment, date of sampling, and pen location on *erm*(B) prevalence and used to determine differences between soil parameters related to *erm*(B) status. Differences were considered significant at $P \leq 0.05$ and were considered tendencies when the P-values ranged from $P = 0.05$ to $P < 0.10$.

RESULTS AND DISCUSSION

The persistence and distribution of the antibiotic resistance gene *erm*(B) was examined in cattle feedlot pens over a 14 month period. Data indicate no differences in the incidence of *erm*(B) over the

Table 1. Prevalence of *erm*(B) positive samples based upon diet fed, pen location, and sample date

		June 2009		August 2010	
Diet*	Pen	Mound†	Edge	Mound	Edge
0% WDGS	307	60.0% (n = 5)‡	71.4% (n = 7)	83.3% (n = 6)	50.0%, (n = 6)
	309	50.0% (n = 4)	100.0% (n = 8)	100.0% (n = 4)	87.5% (n = 8)
	311	80.0% (n = 5)	85.7% (n = 7)	50.0% (n = 2)	70.0% (n = 10)
	313	50.0% (n = 2)	90.0% (n = 10)	75.0% (n = 4)	62.5% (n = 8)
	315	71.4% (n = 7)	100.0% (n = 5)	100.0% (n = 3)	100.0% (n = 9)
	Ave	62.3%	89.4%	81.7%	74.0%
35% WDGS	308	40.0% (n = 5)	85.7% (n = 7)	100.0% (n = 3)	88.9% (n = 9)
	310	25.0% (n = 4)	62.5% (n = 8)	100.0% (n = 4)	87.5% (n = 8)
	312	80.0% (n = 5)	71.4% (n = 7)	75.0% (n = 4)	62.5% (n = 8)
	314	75.0% (n = 4)	75.0% (n = 8)	100.0% (n = 3)	88.9% (n = 9)
	316	83.3% (n = 6)	100.0% (n = 6)	50.0% (n = 4)	87.5% (n = 8)
	Ave	60.7%	78.9%	85.0%	83.1%
0% vs 35% WDGS	P diff	0.905	0.244	0.813	0.404
Overall	Ave	61.5%A§	84.2%B	83.3%B	78.5%B

*Diet indicates either a corn-based diet excluding wet distillers grains plus solubles (0% WDGS) or a diet including up to 35% WDGS.
†Mound indicates sample from the central mound and edge indicates the lower area surrounding the mound.
‡Number of samples in each cell classified as either mound or edge. Twelve total samples per pen.
§Means with different letters within a row are significantly different at $P < 0.05$.

course of the study, regardless of animal diet (Table 1). Initial samples from June 2009 revealed a high prevalence of the gene in the pens, with 76% (n=91) of the samples testing positive for the *erm*(B) gene. In August 2010, fourteen months later, 81% (n=97) of the feedlot surface material samples were positive for the *erm*(B) gene. Thus, despite fourteen months of manure deposition, the prevalence of the gene in the feedlot soil samples showed no statistical change.

Since the initial source of *erm*(B) genes detected in feedlot pen soil is likely to be the fecal deposition, the locations of high manure deposition within the pen were assumed to be the locations where *erm*(B) would most likely be detected. Mapping of the pens with EMI allows for the identification of regions in the pen with signatures characteristic of high manure deposition (Woodbury *et al.*, 2009). Thus, if

the *erm*(B) genes were concentrated in areas of high manure deposition, we would have expected to find more *erm*(B) positive samples in areas with high EMI readings. Our data did not support this theory at either the initial or final sample collection (Figure 1), as *erm*(B) positive and negative results were scattered indiscriminately across low and high EMI reading samples. Pen size and orientation may impact cattle behavior (Wilson *et al.*, 2010), and therefore manure accumulation, so further evaluation of other pens with different designs is warranted.

Next, the ecology of the feedlot pen was considered, including pen design and animal behavior. Cattle feedlot pens are generally outdoors and exposed to the elements. Often there is a mound located in the pen to provide a dry area for the cattle during wet weather (Woodbury *et al.*, 2001). Cattle are non-randomly distributed in the pens and even though cattle movement can mix surface material across the feedlot pen, distinct zones can develop where fecal organisms are fortified (Woodbury *et al.*, 2009). Subtle differences were detected in *erm*(B) gene prevalence between pen sites (mound versus edge) based upon the date (Table 1). Initial prevalence of *erm*(B) on the mound in June 2009 was less than the prevalence of *erm*(B) in pen edge samples in June 2009, and the prevalence differed ($P = 0.016$) from both mound and pen edge samples in August 2010. The prevalence of *erm*(B) in the mound versus the pen edge, however, did not differ from one another in August 2010. A comparison of the overall prevalence in 2009 to 2010 (75.8% and 80.8%, respectively) showed no difference ($P= 0.271$).

A variety of feedlot surface properties were evaluated to determine if any had an effect on *erm*(B) distribution in the cattle feedlot pen (Table 2). Our data did not support the idea that the *erm*(B) genes are distributed across the entire feedlot pen over time. Both *erm*(B) positive and negative surface samples were compared for each sampling date and location (mound versus edge) within the pen. Significant differences were observed between *erm*(B) positive and negative samples for VS, total N, pH, and EC_a. However, for most surface parameters on a particular date and location, there were no differences between *erm*(B) positive and *erm*(B) negative samples. Furthermore, when a significant difference was observed between *erm*(B) positive and negative samples in one set of circumstances (pen location and date), that difference was not significant for any of the other set of date and location combinations. For instance, surface pH for June 2009 in pen edge samples was lower in *erm*(B) positive compared to *erm*(B) negative samples, but there were no differences in pH for these mound or edge samples in August 2010 or in the mound samples for June 2009. There were no clear linkage between *erm*(B) and abiotic environmental parameters of the feedlot surface material such as temperature and pH (Table 2).

In this study, results are based on PCR assays and therefore are not capable of detecting whether viable antibiotic resistant microorganisms are present in the environment, only whether a specific gene is present in the environment. However, since there is concern that antibiotic resistance genes from animal production settings may impact human health via horizontal gene transfer (Brabban *et al.*, 2005; Colomer-Lluch *et al.*, 2011a; b; Hawkey and Jones, 2009), the gene-based information is relevant when considering issues of public health. An organism does not need to be alive to contribute an antibiotic resistance gene. The mechanism used by genes to move through the environment to impact humans remains unclear.

The addition of antibiotic resistant bacteria to the feedlot surface is attributed to animal feces, but after the fecal bacteria leave the gastrointestinal tract (GIT), they are exposed to a drier, more oxygenated soil environment that quickly inactivates or kills many gut microorganisms. The bacterial community found on the feedlot surface material has been shown to be very distinct from the composition of the individual animal's GIT (Durso *et al.*, 2011). So, even though the original source of the *erm*(B) genes is assumed to be fecal bacteria, once excreted from animals the biological components of feces, such as the *erm*(B) genes, display distribution and persistence patterns that are different from those of the chemical and physical components of the fecal material.

Finally, it must be noted that antibiotic resistance

Figure 1. Mapping of feedlot pens at two time points. Results of electromangnetic induction (EMI) maps of feedlot pens are displayed on a color scale. High EMI readings have been previously correlated with areas of high manure deposition (Woodbury *et al.*, 2009; Eigenberg *et al.*, 2010). Results of the *erm*(B) screening locations are displayed using red dots to indicate of *erm*(B) positive samples and black dots to indicate *erm*(B) negative samples.

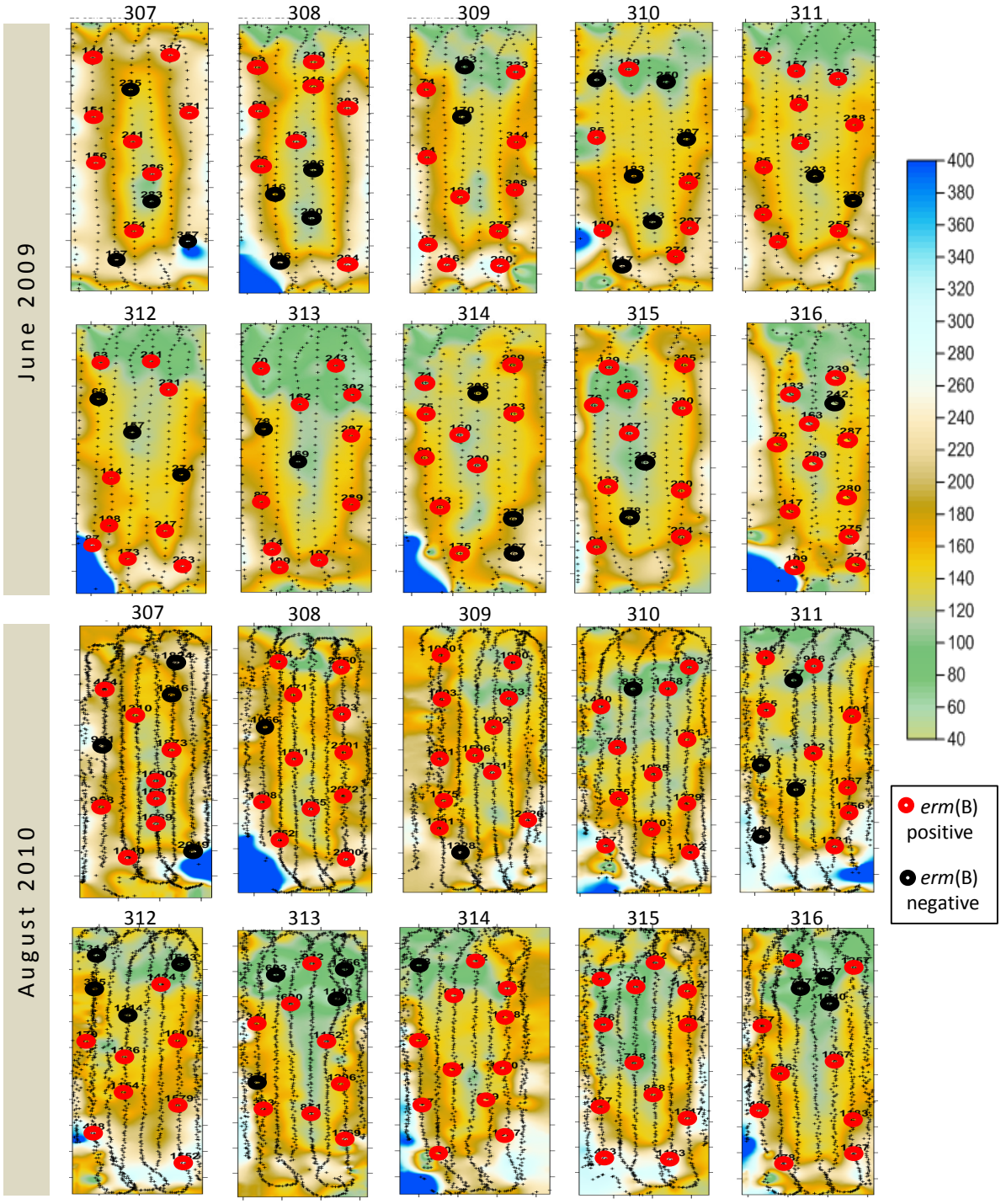

Table 2. Feedlot pen surface composition at the edge and mound areas based upon the detection of *erm*(B) at the beginning and end of the feedlot feeding trial. Bold numbers indicate data that was found to be statistically relevant.

	June 2009		August 2010	
	erm(B) Positive	erm(B) Negative	erm(B) Positive	erm(B) Negative
Pen Edge				
Moisture, %	19.7	17.3	20.3	19.4
Volatile Solids, %	20.1	17.6	17.0	15.1
Total S, g/kg DM	2.4	2.6	2.5	2.3
Total N, g/kg DM	6.7	5.8	**8.4**	**7.2†**
Total P, g/kg DM	3.2	3.1	3.8	3.5
Total K, g/kg DM	9.8	10.3	9.2	8.5
Soil temperature, °C	30.0	28.9	33.2	33.4
Surface temperature, °C	43.3	39.2	43.2	41.3
Soil pH	**7.7**	**8.1***	7.4	7.5
Shallow ECa‡, mS/m	171.2	192.0	**171.1**	**138.8***
Deep ECa, mS/m	165.1	173.6	174.4	149.6
Pen Mound				
Moisture, %	12.3	10.7	14.6	15.2
Volatile Solids, %	13.6	13.0	**13.0**	**9.5***
Total S, g/kg DM	1.4	1.3	**1.7**	**1.3†**
Total N, g/kg DM	5.3	4.4	**6.1**	**4.3***
Total P, g/kg DM	2.3	2.1	**2.8**	**2.2†**
Total K, g/kg DM	8.8	8.2	8.0	6.8
Soil temperature, °C	30.0	30.8	33.1	33.6
Surface temperature, °C	43.5	44.9	42.7	42.6
Soil pH	7.5	7.5	7.3	7.3
Shallow ECa, mS/m	124.5	121.3	119.2	102.0
Deep ECa, mS/m	135.0	134.6	127.6	107.5

*Means with a different letter within a row for a particular sample time differ at $P < 0.05$.

†Indicates a tendency ($0.05<P < 0.1$) for the erm(B) positive and negative samples to differ for that particular sample date.

‡Apparent electrical conductivity as measured by Woodbury *et al.* (2009)

is complex, encompassing many different classes of drugs and mechanisms of resistance. The dynamics of erythromycin resistance, as coded for by *erm*(B), is not necessarily the same as the dynamics of other macrolide antibiotic resistance genes. There is not currently enough information to determine how different kinds of antibiotic resistance genes persist and move through agroecosystems, or how the data collected here for *erm*(B) relates to distribution and persistence of other antibiotic resistance genes. Previous studies strongly support the idea that the composition of resistance genes in any particular habitat is a reflection of the species of bacteria that are commonly found in each environment (Durso *et al.*, 2012; Patterson *et al*., 2007).

In conclusion, *erm*(B) genes were not enriched in feedlot soils despite 14 months of manure accumulation. Locations of high manure deposition were not the same as the locations of the *erm*(B) gene and the gene was not associated with specific feedlot pen zones. The dynamics of antibiotic resistance in cattle feedlot pens is likely dependent on the specific antibiotic resistance gene being studied, and is likely influenced by a number of biological, physical, and chemical parameters of the soil.

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REFERENCES

Alexander, T.W., J.L. Yanke, T. Reuter, E. Topp, R.R. Read, B.L. Selinger, and T.A. McAllister. 2011. Longitudinal characterization of antimicrobial resistance genes in feces shed from cattle fed different subtherapeutic antibiotics. BMC Microbiol. 11:19.

Anderson, J.F., T.D. Parrish, M. Akhtar, L. Zurek, and H. Hirt. 2008. Antibiotic resistance of enterococci in American Bison (*Bison bison*) from a nature preserve compared to that of enterococci in pastured cattle. Appl. Environ. Microbiol. 74:1726-1730.

Bae, W., K.N. Kaya, D.D. Hancock, D.R. Call, Y.H. Park, and T.E. Besser. 2005. Prevalence and antimicrobial resistance of thermophilic *Campylobacter* spp. from cattle farms in Washington state. Appl. Environ. Microbiol. 71:169-174.

Böckelmann, U., H.H. Dörries, M.N. Ayuso-Gabella, M. Salgot de Marçay, V. Tandoi, C. Levantesi, C. Masciopinto, E. Van Houtte, U. Szewzyk, T. Wintgens, and E. Grohman. 2009. Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. Appl. Environ. Microbiol. 75:154-163.

Brabban, A.D., E. Hite, and T.R. Callaway. 2005. Evolution of foodborne pathogens via temperate bacteriophage-mediated gene transfer. Foodborne Pathog. Dis. 2, 287-303.

Chen, J, Z. Z. Yu, F.C. Michel, Jr., T. Wittum, and M. Morrison. 2007. Development and application of Real-time PCR assays for quantification of *erm* genes conferring resistance to macrolides-lincosamides-sterptogramin B in livestock manure and manure management systems. Appl. Environ. Microbiol. 73:4407-4416.

Colomer-Lluch, M., L. Imamovic, J. Jofre, and M. Muniesa. 2011a. Bacteriophages carrying antibiotic resistance genes in fecal waste from cattle, pigs, and poultry. Antimicrob. Agents Chemother. 55:4908–4911. doi: 10.1128/AAC.00535-11.

Colomer-Lluch, M., J. Jofre, and M. Muniesa. 2011b. Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. PLoS One 6, e17549. doi: 10.1371/journal.pone.0017549.

De Leener, E., A. Martel, A. Decostere, and F. Haesebrouck. 2004. Distribution of the *erm* (B) gene, tetracycline resistance genes, and Tn1545-like transposons in macrolide- and lincosamide-resistant enterococci from pigs and humans. Microb. Drug Resist. 10:341-345.

Dogan, B., Y. H. Schukken, C. Santisteban, and K. J. Boor. 2005. Distribution of serotypes and antimicrobial resistance genes among *Streptococcus agalactiae* isolates from bovine and human hosts.

J. Clin. Microbiol. 43:5899-5906.

Durso, L.M., G.P. Harhay, T.P. Smith, J.L. Bono, T.Z. DeSantis, and M.L. Clawson. 2011. Bacterial community analysis of beef cattle feedlots reveals that pen surface is distinct from feces. Foodborne Pathog. Dis. 8:647-649. doi: 10.1089/fpd.2010.0774.

Durso, L.M., D.N. Miller, and B.J. Wienhold. 2012. Distribution and quantification of antibiotic resistant genes and bacteria across agricultural and non-agricultural metagenomes. PLOS ONE 10.1371/journal.pone.0048325

Eigenberg,, R.A., B.L. Woodbury, J.A. Nienaber, M.J. Spiehs, D.B. Parker, and V.H. Varel. 2010. Soil conductivity and multiple linear regression for precision monitoring of beef feedlot manure and runoff. J. Environ. & Eng. Geophys. 15:175-183.

Hawkey, P.M. and A.M. Jones. 2009. The changing epidemiology of resistance. J Antimicrob Chermother 64:i3-i10. doi: 10.1093/jac/dkp256.

Isaacson, R.E. and M.E. Torrence. 2002. The role of antibiotics in agriculture. American Academy of Microbiology, Washington, DC.

Jacob, M.E., Z.D. Paddock, D.G. Renter, K.F. Lechtenberg, and T.G. Nagaraja. 2010. Inclusion of dried or wet distillers' grains at different levels in diets of feedlot cattle affects fecal shedding of *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 76:7238-7242. doi: 10.1128/AEM.01221-10.

Jost, B. H., H. T. Trinh, J. G. Songer, and S. J. Billington. 2004. A second tylosin resistance determinant, *Erm* B, in *Arcanobacterium pyogenes*. Antimicrob. Agents Chemother. 48:721-727.

Lesch, S.M., J.D. Rhoades, and D.L. Corwin. 2000. ESAP-95 version 2.10R: User manual and tutorial guide. Research Rep. 146. USDA–ARS, G.E. Brown, Jr. Salinity Laboratory, Riverside, CA.

Miller, D.N., J.E. Bryant, E.L. Madsen, and W.C. Ghiorse. 1999. Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl. Environ. Microbiol. 65:4715-4724.

Patterson, A. J., R. Colangeli, P. Spigaglia, and K.P. Scott. 2007. Distribution of specific tetracycline and erythromycin resistance genes in environmental samples assessed by macroarray detection. Environ. Microbiol. 9: 703–715. doi: 10.1111/j.1462-2920.2006.01190.x

Roberts, M.C., J. Sutcliffe, P. Courvalin, L.B. Jenses, J. Rood, and H. Seppala. 1999. Nomenclature for macrolide and macrolide-lincosamide-stretogramin B resistance determinants. Antimicrob. Agents Chemother. 43:2823-2830.

Roberts, M.C. 2004. Distribution of macrolide, lincosamide, streptogramin, ketolide and oxazolidinone (MLSK) resistance genes in Gram-negative bacteria. Curr. Drug Targets Infect. Disord. 4:207-215.

Smith Thomas, H. 2009. The Cattle Health Handbook: preventative care, disease, treatments and emergency procedures. Editors R. Boyd-Owens, S. Guare, D. Burns. Storey Publishing, North Adams, MA , pp38-39.

Spiehs, M.J., D.N. Miller, B.L. Woodbury, R.A. Eigenberg, V.H. Varel, and D.B. Parker. 2012. Effect of feeding wet distillers grains with solubles to beef cattle on air and manure quality. Appl. Eng. Agricul. 28:423-430.

Unc, A. and M.J. Goss. 2004. Transport of bacteria from manure and protection of water resources. Appl. Soil Ecol. 25:1-18.

Wilson, S.C., R.C. Dobos, and L.R. Fell. 2010. Spectral analysis of feeding and lying behavior of cattle kept under different feedlot conditions. J. Appl. Anim. Welfare Sci. 8:13-24.

Woodbury, B.L., D.N. Miller, J.A. Nienaber, and R.A. Eigenberg. 2001. Seasonal and spatial variations of denitrifying enzyme activity in feedlot soil. Trans. ASAE 44:1635-1642.

Woodbury, B.L., S.M. Lesch, R.A. Eigenberg, D.N. Miller, and M.J. Spiehs. 2009. Electromagnetic induction sensor data to identify areas of manure accumulation on a feedlot surface. Soil Sci. Soc. Am. J. 73:2068-2077.

World Health Organization. 2007. Critically important antimicrobials for human medicine : categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use : report of the second WHO Expert Meeting, Copenhagen, 29-31 May. http://www.who.int/foodborne_disease/resistance/antimicrobials_human.pdf. Accessed May, 2013.